

Figure 1

SEQ ID NO:

6	mouse_E3 $\alpha$ II	MASEMEPEVQ AID-RSLLEC SAEEIAGRWL QATDLNREVY QHLAHCPKI	49
4	human_E3 $\alpha$ II	MASELEPEVQ AID-RSLLEC SAEEIAGKWL QATDLTREVY QHLAHYVPKI	49
15	mouse_E3 $\alpha$ I	MADEEMDGAE RMDVSPEPPL APQRPASWWD QQVDFYTAFL HHLAQLVPEI	50
2	human_E3 $\alpha$ I	MADEEAGGTE RMEISAEQPQ TPQRLASWWD QQVDFYTAFL HHLAQLVPEI	50
	Consensus	MA.E.....D....L...A.W.Q..D.....HLA..VP.I	50
6	mouse_E3 $\alpha$ II	YCRGPNPFPQ KEDTLAQHIL LGPMEWYICA EDPALGFPL EQANKPSHLC	99
4	human_E3 $\alpha$ II	YCRGPNPFPQ KEDMLAQHVL LGPMEWYLCG EDPAFGFPL EQANKPSHLC	99
15	mouse_E3 $\alpha$ I	YFAEMDPDLE KQEESVQMSI LTPLEWYLFQ EDPDICLEKL KHSG-AFQLC	99
2	human_E3 $\alpha$ I	YFAEMDPDLE KQEESVQMSI FTPLEWYLFQ EDPDICLEKL KHSG-AFQLC	99
	Consensus	Y.....P...K.....Q...L.P.EWYL.G EDP.....KL .....LC	100
6	mouse_E3 $\alpha$ II	GRVFKVGEPT YSCRDCAVDP TCVL.CMECFL GSIHRDHRYR MTTSGGGFC	149
4	human_E3 $\alpha$ II	GRVFKVGEPT YSCRDCAVDP TCVL.CMECFL GSIHRDHRYR MTTSGGGFC	149
15	mouse_E3 $\alpha$ I	GKVKFKSGETT YSCRDCAIIDP TCVL.CMDCFQ SSVHKHNRYK MHTSTGGGFC	149
2	human_E3 $\alpha$ I	GRVFKFKSGETT YSCRDCAIIDP TCVL.CMDCFQ DSVHKHNRYK MHTSTGGGFC	149
	Consensus	GRVFK.GE.T YSCRDC.A.DP TCVL.CM.CF. .S.H..HRY. M.TS.GGGFC	150
6	mouse_E3 $\alpha$ II	DCGDTEAWKE GPYCQKHMLS SSEVVEEEEDP LVHLSEDVIA RTYNIFAIMF	199
4	human_E3 $\alpha$ II	DCGDTEAWKE GPYCQKHELN TSEIEEEEEDP LVHLSEDVIA RTYNIFAIMF	199
15	mouse_E3 $\alpha$ I	DCGDTEAWKT GPFCVDHEPG RAGTTKESLH -CPLNEEVIA QARRIFPSVI	198
2	human_E3 $\alpha$ I	DCGDTEAWKT GPFCVNHEPG RAGTIKENSR -CPLNEEVIV QARKIFPSVI	198
	Consensus	DCGDTEAWK. GP.C..HE...E...L.E.VIA ....IF....	200
6	mouse_E3 $\alpha$ II	RYAVDILTWE KESELPEDLE VAEKS'DTYYC MLFNDEVHTY EQVIYTLQKA	249
4	human_E3 $\alpha$ II	RYAVEILTWE KESELPADLE MVEKS'DTYYC MLFNDEVHTY EQVIYTLQKA	249
15	mouse_E3 $\alpha$ I	KYIVEMTIWE EEKELPPELQ IREKNERYYYC VLFNDEHHSY DHVIYSLQRA	248
2	human_E3 $\alpha$ I	KYVVEMTIWE EEKELPPELQ IREKNERYYYC VLFNDEHHSY DHVIYSLQRA	248
	Consensus	.Y.VE...WE .E.ELP..L. ..EK...YYC .LFNDE.H.Y ..VIY..LQ.A	250
6	mouse_E3 $\alpha$ II	VNCTQKEAIG FATTVDRDGR RPVRYGDFQY CDQAKTVIVR NTSRQTK-PL	298
4	human_E3 $\alpha$ II	VNCTQKEAIG FATTVDRDGR RSVRYGDFQY CEQAKSVIVR NTSRQTK-PL	298
15	mouse_E3 $\alpha$ I	LDCELAEAQL HTTAIDKEGR RAVKAGVYAT CQEAKEDIKS HSENVSQHPL	298
2	human_E3 $\alpha$ I	LDCELAEAQL HTTAIDKEGR RAVKAGAYAA CQEAKEDIKS HSENVSQHPL	298
	Consensus	..C...EA...T..D..GR R.V..G....C..AK..I...PL	300
6	mouse_E3 $\alpha$ II	KVQVMHSSVA AHQNFGLKAL SWLGSTIGYS DGLRRILCQV GLQEGPDGEN	348
4	human_E3 $\alpha$ II	KVQVMHSSIV AHQNFGLKLL SWLGSTIGYS DGLRRILCQV GLQEGPDGEN	348
15	mouse_E3 $\alpha$ I	HVEVLHSVVM AHQKFALRLG SWMNKIMSYS SDFRQIFCQA CLVEEPGSEN	348
2	human_E3 $\alpha$ I	HVEVLHSEIM AHQKFALRLG SWMNKIMSYS SDFRQIFCQA CLREEPDSEN	348
	Consensus	.V.V.HS... AHQ.F.L.L. SW...I..YS ...R.I.CQ. .L.E.PD.EN	350
6	mouse_E3 $\alpha$ II	SSLVDRLMLN DSKLWKGARS VYHQLFMSSL LMDLKYYKKLF ALRFAKNYRQ	398
4	human_E3 $\alpha$ II	SSLVDRLMLS DSKLWKGARS VYHQLFMSSL LMDLKYYKKLF AVRFAKNYQQ	398
15	mouse_E3 $\alpha$ I	PCLISRLMLW DAKLYKGARK ILHELIFSSF FMEMEYKKLF AMEFVKYYKQ	398
2	human_E3 $\alpha$ I	PCLISRLMLW DAKLYKGARK ILHELIFSSF FMEMEYKKLF AMEFVKYYKQ	398
	Consensus	..L..RLML. D.KL.KGAR. ..H.L..SS. .M...YKKLF A..F.K.Y.Q	400
6	mouse_E3 $\alpha$ II	LQRDFMEDDH ERAVSVTALS VQFFTAPTLA RMLLTEENLM TVIKAFTMDH	448
4	human_E3 $\alpha$ II	LQRDFMEDDH ERAVSVTALS VQFFTAPTLA RMLITEENLM SIIIKTFMDH	448
15	mouse_E3 $\alpha$ I	LQKEYISDDH ERSISITALS VQMLTVPTLA RHLIEEQNVI SVITETLLEV	448
2	human_E3 $\alpha$ I	LQKEYISDDH DRSSISITALS VQMFTVPTLA RHLIEEQNVI SVITETLLEV	448
	Consensus	LQ.....DDH ER..S.TALS VQ.FT.PTLA R.LI.E.N.. SVI..T...	450

Figure 1 (continued)

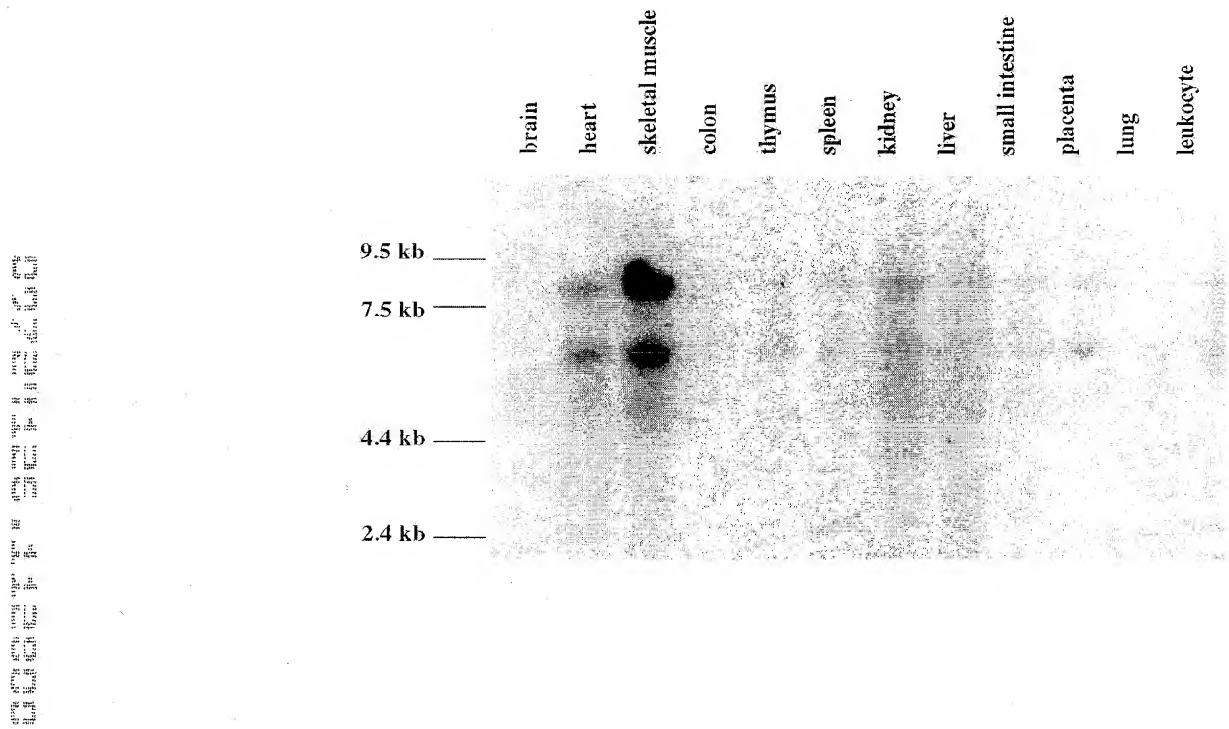
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6	mouse_E3αII	LKHRDAQGRF QFERYTALQA FKFRRVQSLI LDLKYVLISK PTEWSDELRO	498
4	human_E3αII	LRHRDAQGRF QFERYTALQA FKFRRVQSLI LDLKYVLISK PTEWSDELRO	498
15	mouse_E3αI	LPEYLDRNN- KFN-FQGYSQ DKLGRVYAVI CDLKYILISK PVIWTERLRA	496
2	human_E3αI	LPEYLDRNN- KFN-FQGYSQ DKLGRVYAVI CDLKYILISK PTIWTERLRM	496
	Consensus	L..... F..... K..RV... I .DLKY.LISK PT.W...LR.	500
6	mouse_E3αII	KFLQGFDAFL ELLKCMQGMD PITRQVGQHI EMEPEWAAF TLQMKLTHVI	548
4	human_E3αII	KFLEGFDAFL ELLKCMQGMD PITRQVGQHI EMEPEWAAF TLQMKLTHVI	548
15	mouse_E3αI	QFLEGFRSFL KILTCMQGME EIRRQVGQHI EVDPDWEAAI AIQMQLKNIL	546
2	human_E3αI	QFLEGFRSFL KILTCMQGME EIRRQVGQHI EVDPDWEAAI AIQMQLKNIL	546
	Consensus	.FLEGF..FL ..L.CMQGM. .I.RQVGQHI E..P.WEAA. ..QM.L....	550
6	mouse_E3αII	SMVQDWCAKD EKVLIEAYKK CLAVLTQCHG GFTDGEQPIT LSICGHSVET	598
4	human_E3αII	SMMQDWCAASD EKVLIEAYKK CLAVLMQCHG GYTDGEQPIT LSICGHSVET	598
15	mouse_E3αI	LMFQEWCACD EDLLLVAYKE CHKAVMRCST NFMSSTKTV- VQLCGHSLET	595
2	human_E3αI	LMFQEWCACD EELLLVAYKE CHKAVMRCST SFISSSKTV- VQSCGHSLET	595
	Consensus	:M.Q.WCA.D E..L..AYK. C....M.C.. .F..... .CGHS.ET	600
6	mouse_E3αII	IRYCVSQEKV SIHLPISRLL AGLHVLLSKS EVAYKFPELL PLSELSPPML	648
4	human_E3αII	IYCVSQEKV SIHLPVSRLL AGLHVLLSKS EVAYKFPELL PLSELSPPML	648
15	mouse_E3αI	KSYKVSEDLV SIHLPLSRTL AGLHVRLSRL GAISRLHEFV PFDSFQVEVL	645
2	human_E3αI	KSYRVSEDLV SIHLPLSRTL AGLHVRLSRL GAVSRLHEFV SFEDFQVEVL	645
	Consensus	..Y.VS...V SIHLPL.SR.L AGLHV.LS.. ....E.. P.....L	650
6	mouse_E3αII	IEHPLRCLVL CAQVHAGMWR RNGFSLVNQI YYYHNVKCRR EMFDKDIVML	698
4	human_E3αII	IEHPLRCLVL CAQVHAGMWR RNGFSLVNQI YYYHNVKCRR EMFDKDVVML	698
15	mouse_E3αI	VEYPLRCLVL VAQVVAEMWR RNGLSLISQV FYYQDVVKCRE EMYDKDIIML	695
2	human_E3αI	VEYPLRCLVL VAQVVAEMWR RNGLSLISQV FYYQDVVKCRE EMYDKDIIML	695
	Consensus	.E.PLRCLVL .AQV.A.MWR RNG.SL..Q. .YY..VKCR. EM.DKDI.ML	700
6	mouse_E3αII	QTGVSMMDPN HFLMIMLSRF ELYQLFSTPD YGKRFSSEVT HKDVVQQNNT	748
4	human_E3αII	QTGVSMMDPN HFLMIMLSRF ELYQIFSTPD YGKRFSSEIT HKDVVQQNNT	748
15	mouse_E3αI	QIGASIMDPN KFLLLVLQRY EL----TDA FNKTIST--K DQDLIKQYNT	738
2	human_E3αI	QIGASLMDPN KFLLLVLQRY EL----AEA FNKTIST--K DQDLIKQYNT	738
	Consensus	Q.G.S.MDPN .FL...L.R. EL....T.. .K..S.... .D...Q.NT	750
6	mouse_E3αII	LIEEMLYLI MGVGERFNPG VGQVAATDEI KREIIHQLSI KPMMAHSELVK	798
4	human_E3αII	LIEEMLYLI MGVGERFSPG VGQVNATDEI KREIIHQLSI KPMMAHSELVK	798
15	mouse_E3αI	LIEEMLQVLI YIVGERYVPG VGNVTREEVI MREITHLLCI EPMPHSAIAR	788
2	human_E3αI	LIEEMLQVLI YIVGERYVPG VGNVTKEEVN MREIIHLLCI EPMPHSAIAK	788
	Consensus	LIEEML...I ..VGER..PG VG.V.....I .REIIH.L.I .PM.HS...K	800
6	mouse_E3αII	SLPEDENKET GMESVIESVA HFKKPGLTGR GMYELKPECA KEFNLYFYHF	848
4	human_E3αII	SLPEDENKET GMESVIEAVA HFKKPGLTGR GMYELKPECA KEFNLYFYHF	848
15	mouse_E3αI	NLPENENNNT GLENVINKVA TFKKPGVSGH GVYELKDESL KDFNMYFYHY	838
2	human_E3αI	NLPENENNNT GLENVINKVA TFKKPGVSGH GVYELKDESL KDFNMYFYHY	838
	Consensus	.LPE.EN.ET G.E.VI..VA .FKKPG..G. G.YELK.E...K.FN.YFYH.	850
6	mouse_E3αII	SRAEQSKAEE AQRKLKRENK EDTALPPPALL PPFCPLFASL VNILQCDVML	898
4	human_E3αII	SRAEQSKAEE AQRKLKRQNR EDTALPPPVL PPFCPLFASL VNILQSDVML	898
15	mouse_E3αI	SKTQHSKAEH MQKRRRKQEN KDEALPPPPP PEFCPAFSKV VNLLSCDVM	888
2	human_E3αI	SKTQHSKAEH MQKRRRKQEN KDEALPPPPP PEFCPAFSKV INLLNCDIMM	888
	Consensus	S....SKAE. .Q.K...Q.. .D.ALPPP.. P.FCP.F... VN.L.CDVM.	900

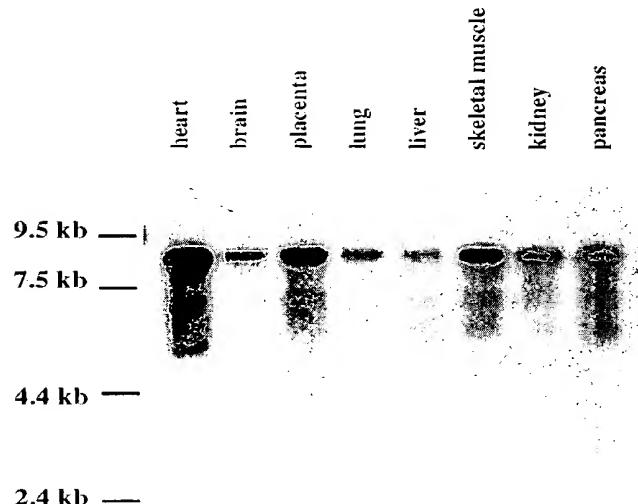
Figure 1 (continued)

Figure 1 (continued)

**Figure 2**  
**The Expression Profile of huE3 $\alpha$ -II in Human Tissues**



**Figure 3**  
**The Expression Profile of huE3 $\alpha$ -I in Human Tissues**



**Figure 4- Ubiquitination of Endogenous Proteins**

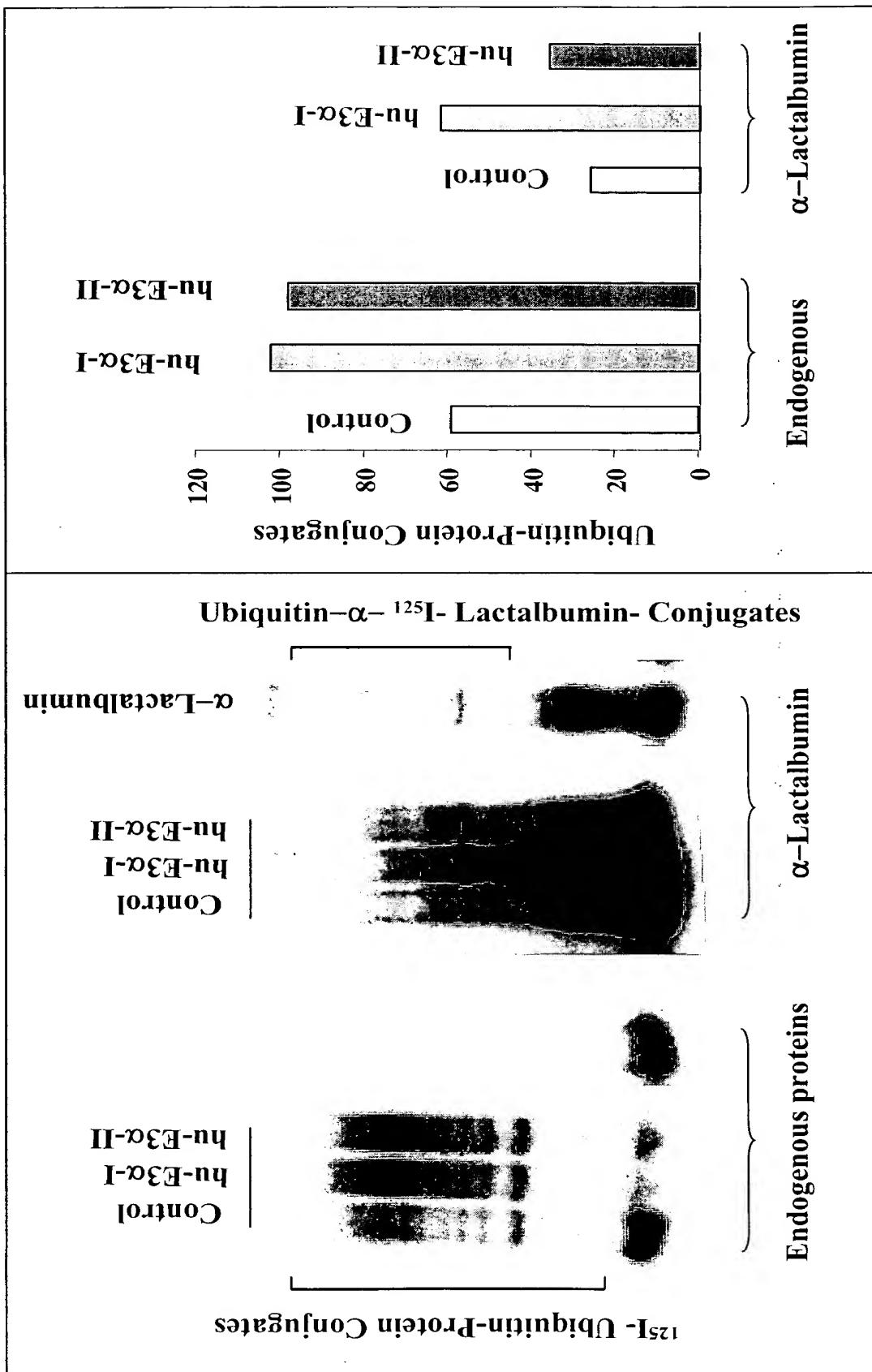
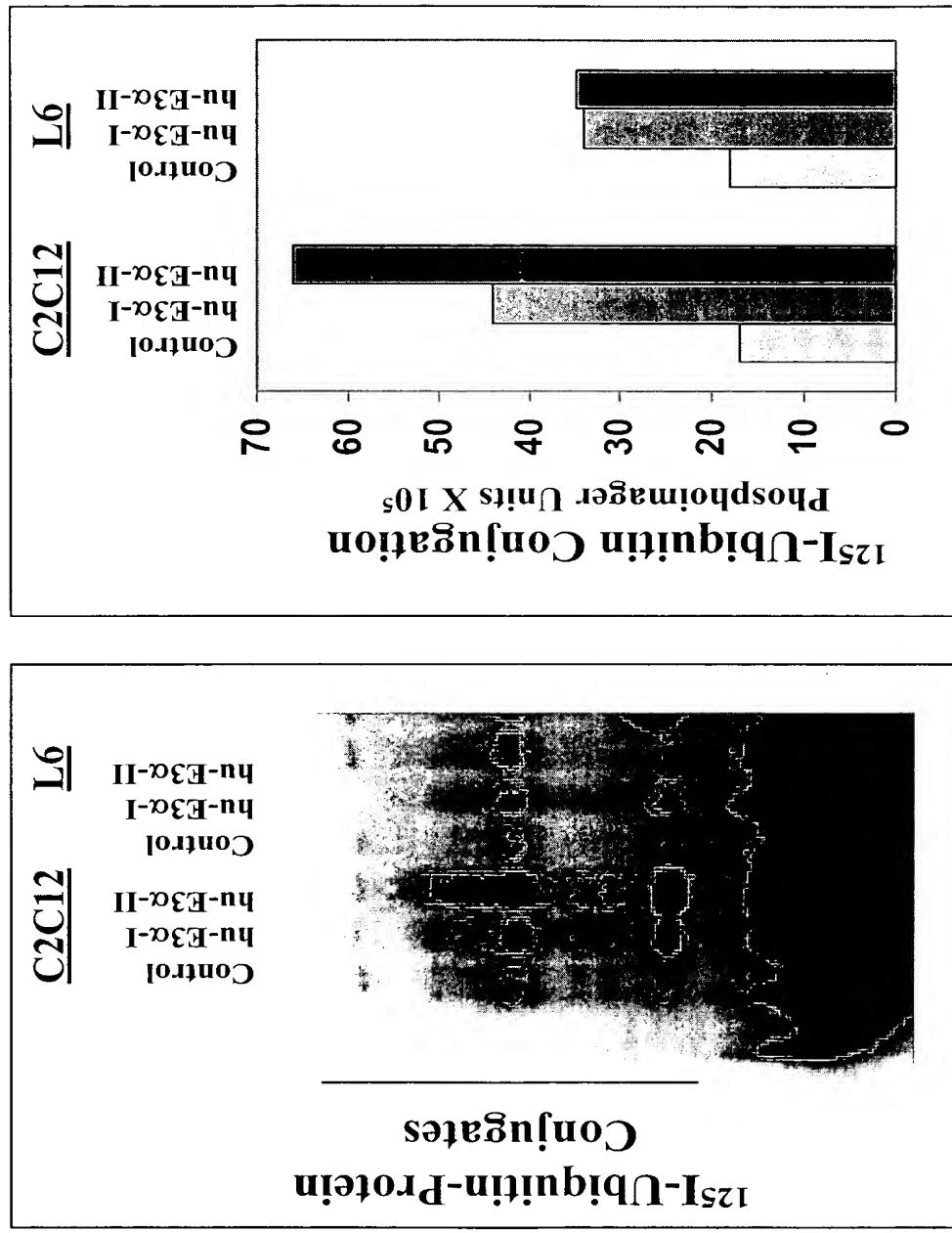


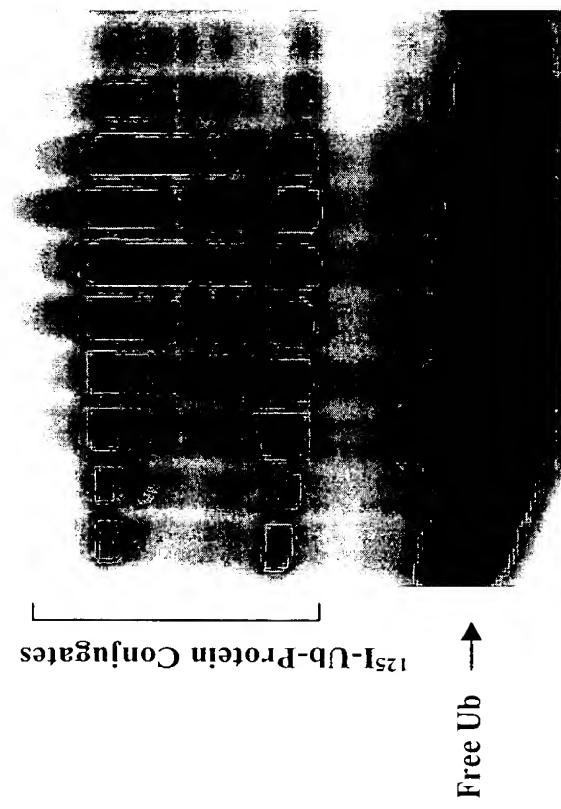
Figure 5

Transfection of Human E3a-I or E3a-II cDNA Stimulates  
Ubiquitin Conjugation in Cultured Muscle Cell Lines



**Figure 6** **<sup>125</sup>I-Ubiquitin Conjugation to Muscle Proteins and Its Sensitivity to E3 $\alpha$  Inhibitor in Skeletal Muscle Extracts**

Contro v.s. 3-day tumor-bearing



Contro v.s. 5-day tumor-bearing

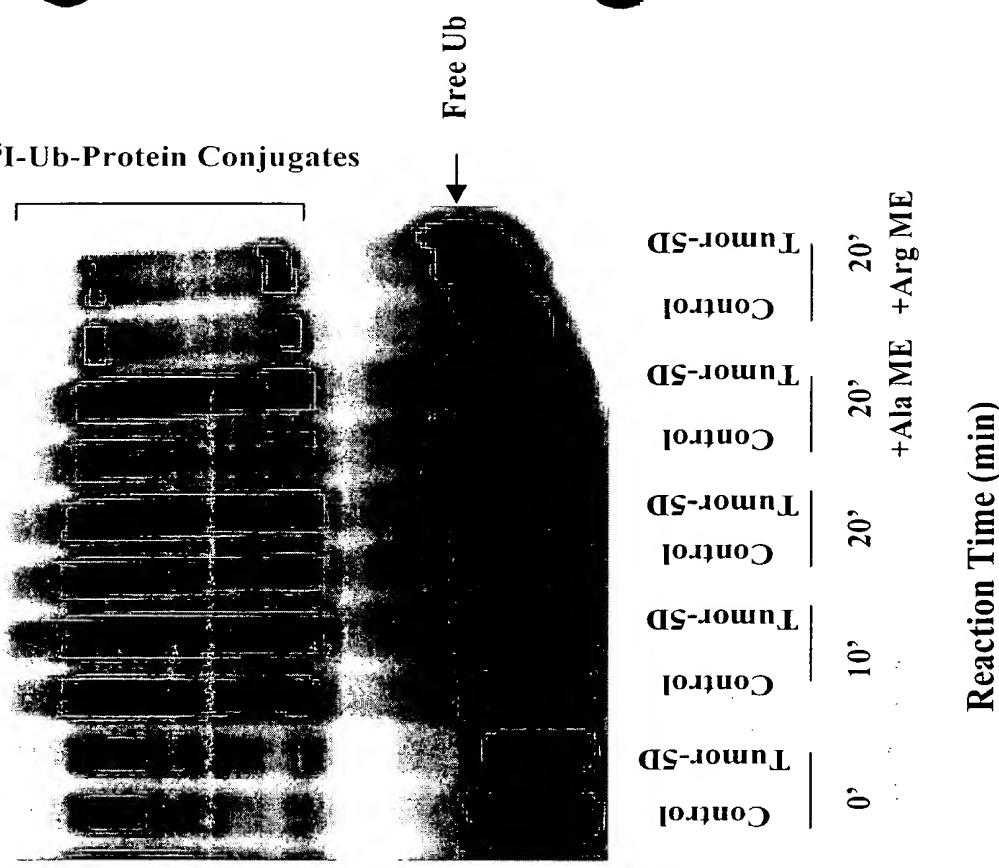
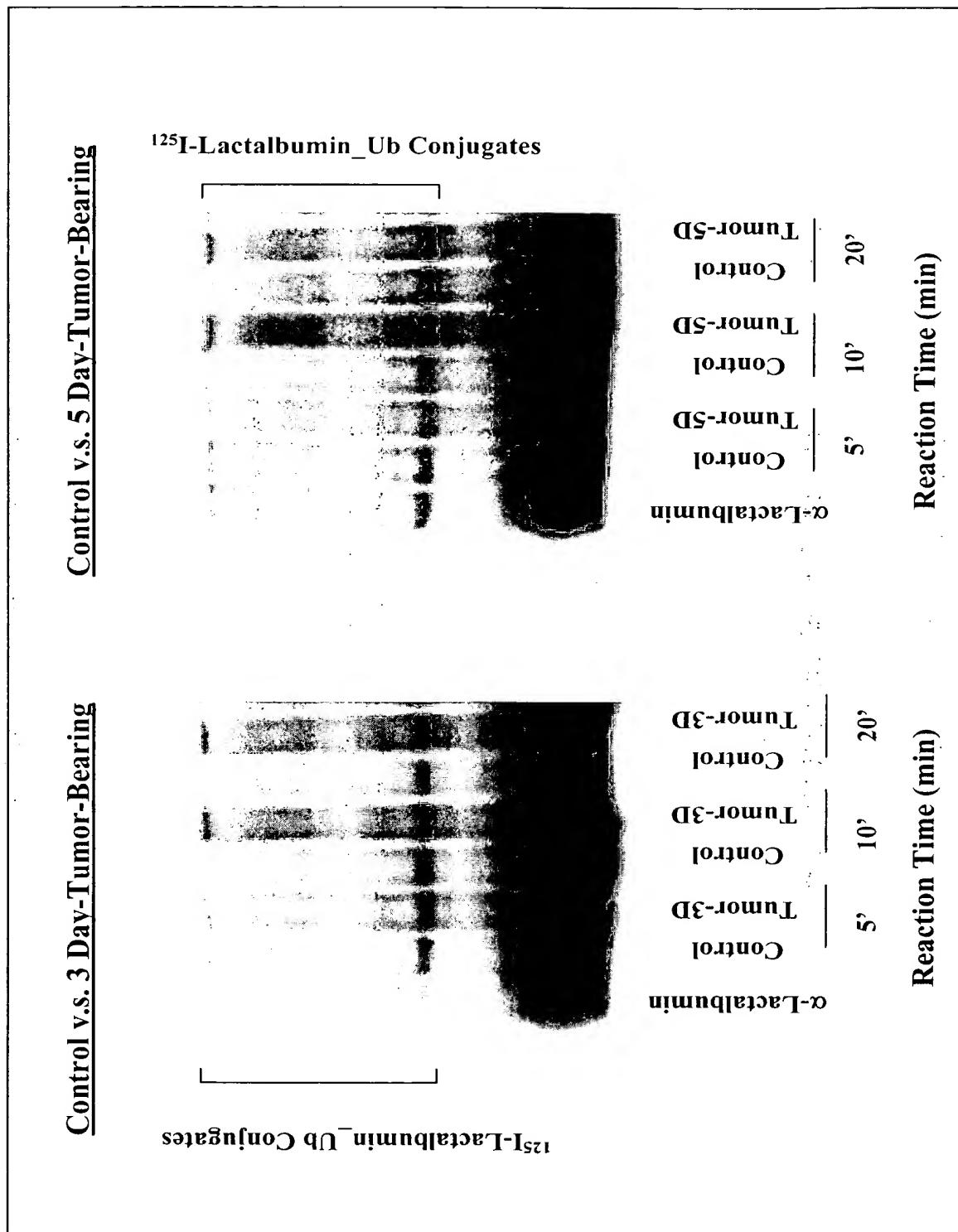


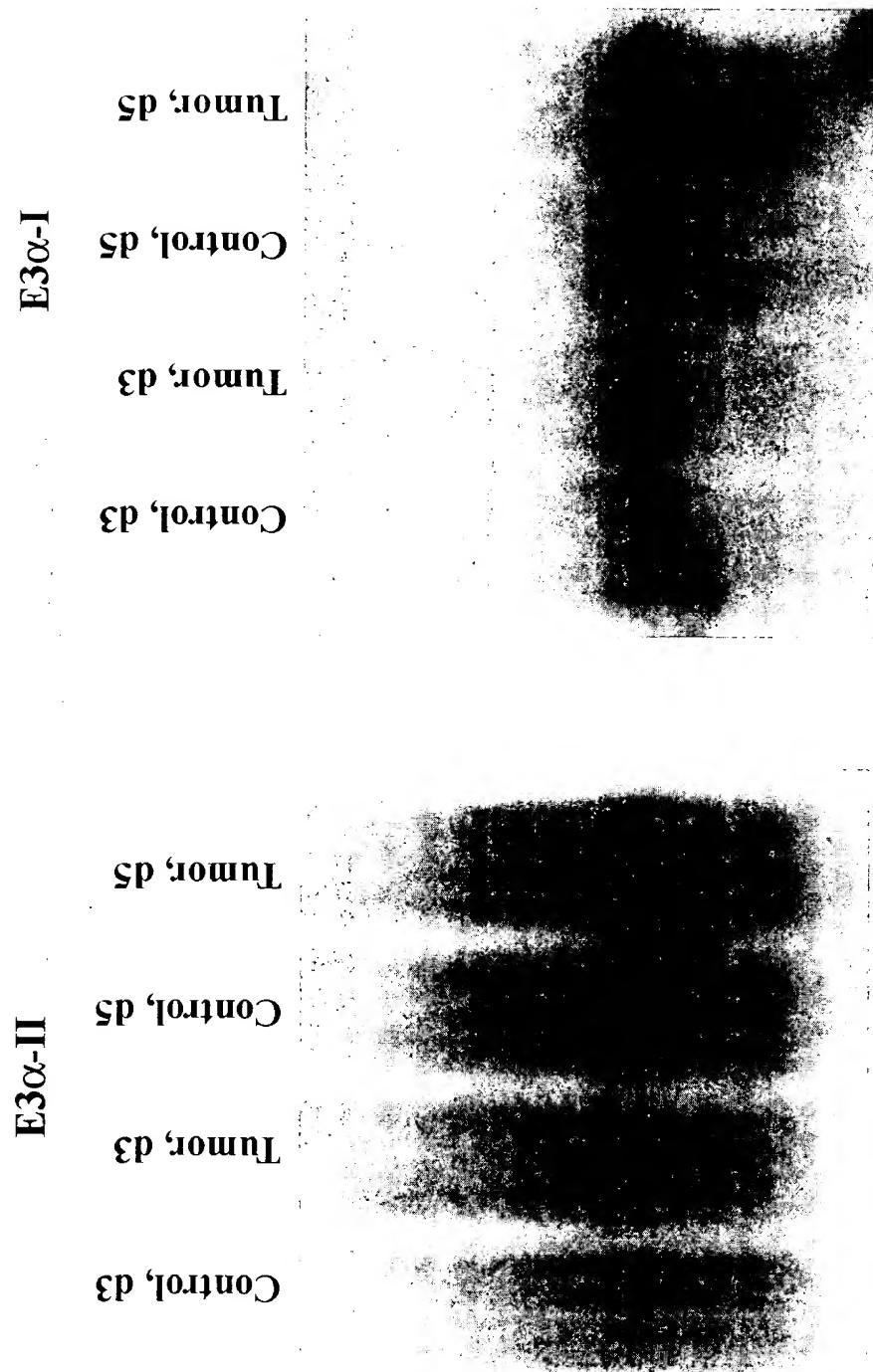
Figure 7

# Rates of Ubiquitination of N-end Rule Substrate $\alpha$ -Lactalbumin in Skeletal Muscle Extracts



**Figure 8**

**Northern blot analysis of E3 $\alpha$ -I & E3 $\alpha$ -II expression  
in gastrocnemius muscles in YAH-130 experimental cachexia model**



**Figure 9**

**Northern blot analysis of E3 $\alpha$ -I and E3 $\alpha$ -II expression in gastrocnemius muscle and cardiac muscle in C26 experimental cachexia model**

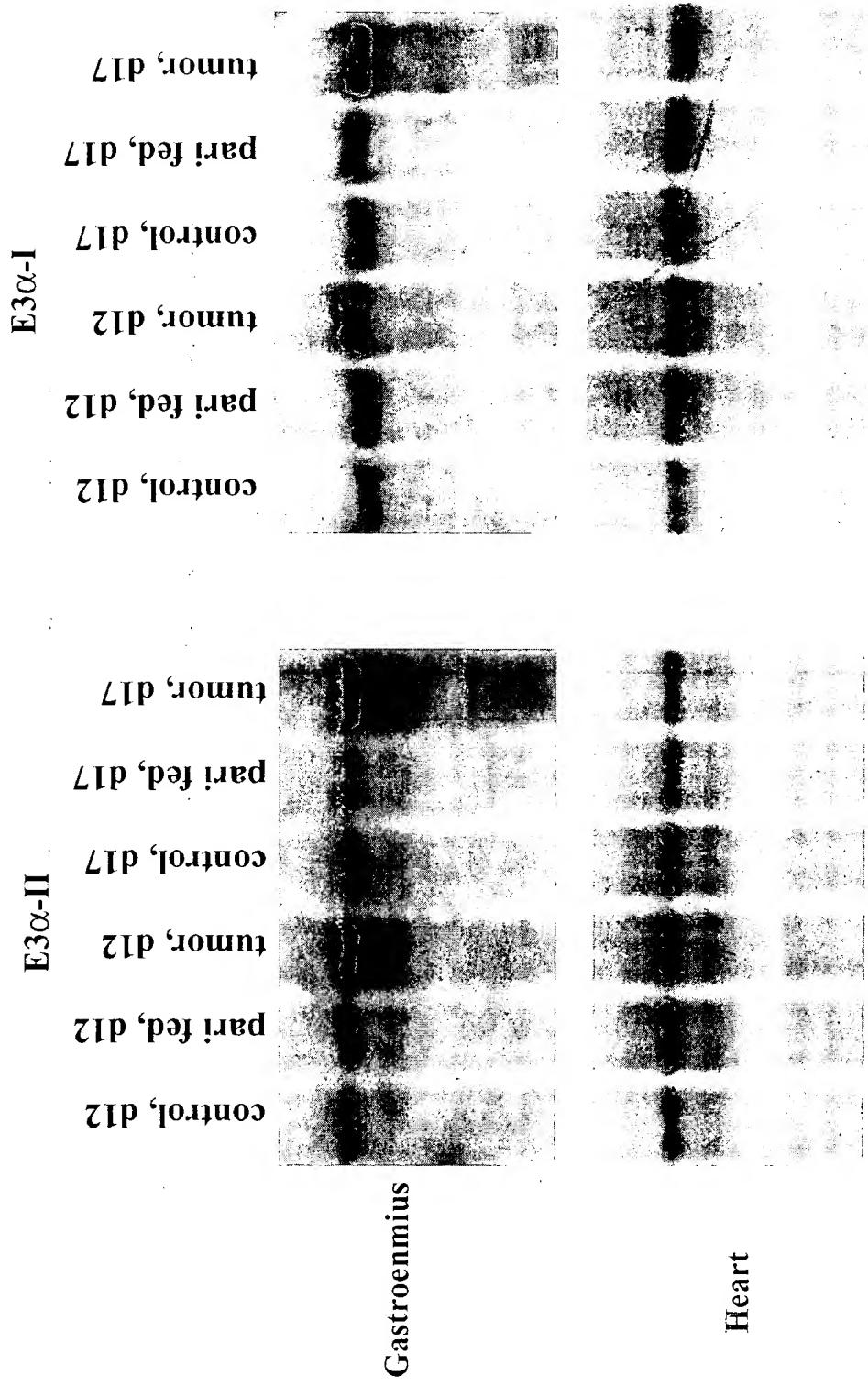


Figure 10

Proinflammatory cytokines TNF- $\alpha$  and IL-6  
induce E3 $\alpha$ -II Expression in C2C12 myotube culture

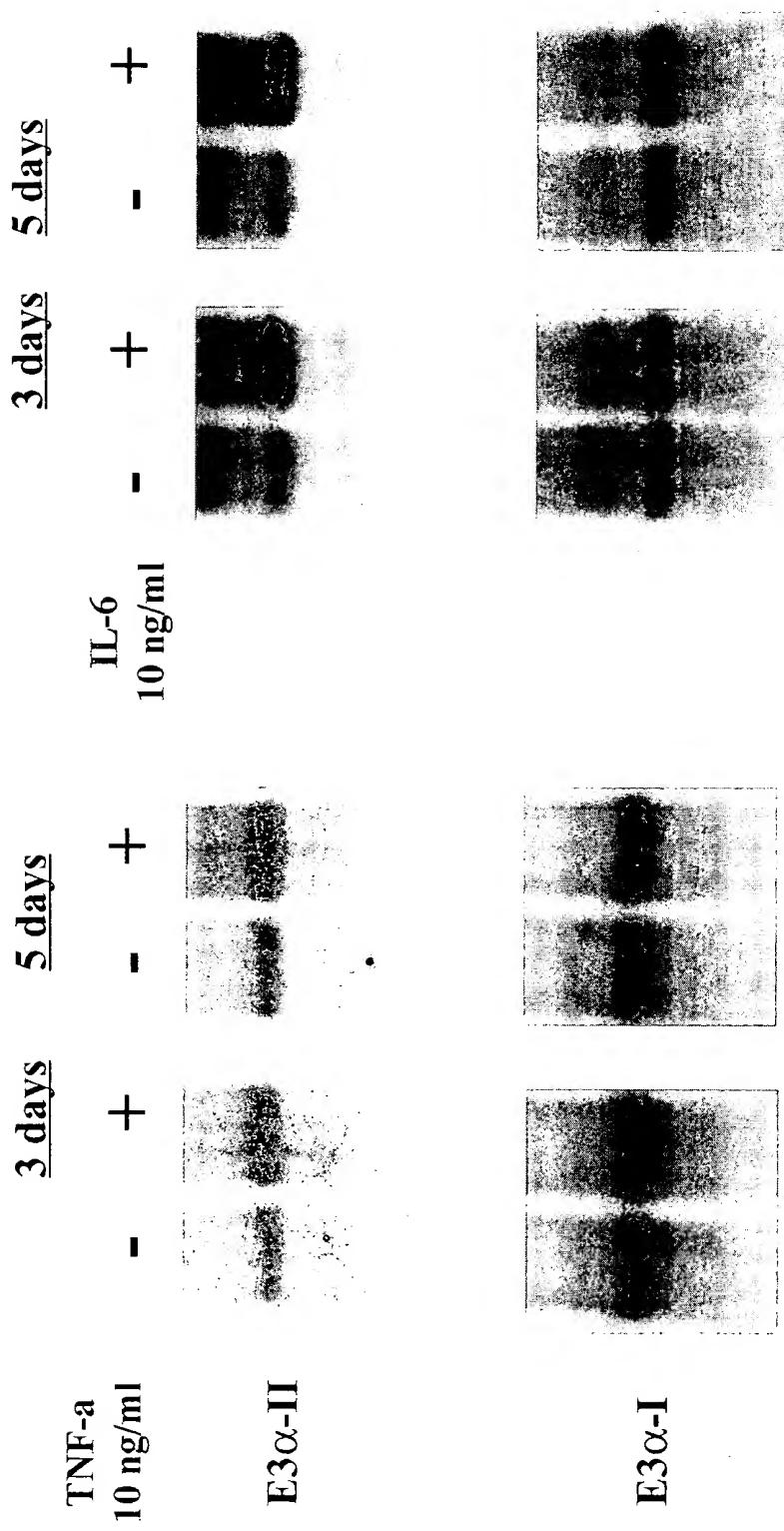
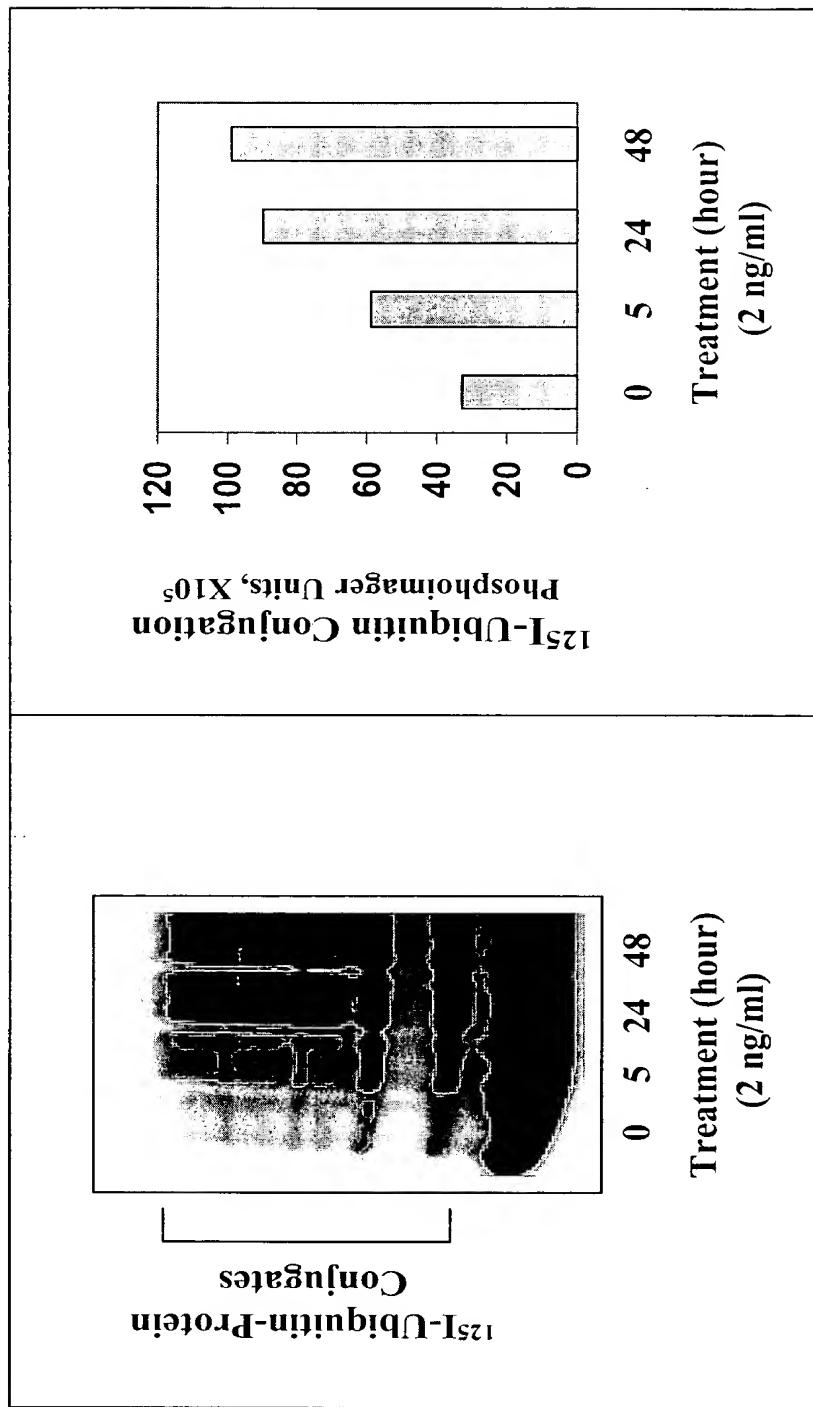


Figure 11

IL-6 Elicits Accelerated Ubiquitination in C2C12 Myotube Cultures



**Figure 12**

**TNF $\alpha$  Elicits Accelerated Ubiquitination in C2C12 Myotube Cultures**

